PATENT
Customer No. 22,852
Attorney Docket No. 03495.0202

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Gouzel KARIMOVA et al.	)	Group Art Unit: Not Assigned
	)	
Serial No.: 09/818,939	)	Examiner: Not Assigned
	)	
Filed: March 28, 2001	)	
	)	
For: IMPROVEMENTS TO A BACTERIAL TWO-HYBRID SYSTEM FOR PROTEIN-PROTEIN INTERACTON SCREENING, NEW STRAINS FOR USE THEREIN, AND THEIR APPLICATIONS	)	

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09818939 032801

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the above application, please amend this application as follows:

IN THE SPECIFICATION:

On page 5, line 6. after "NewSfi" insert --[SEQ ID No.6]--

On page 5, line 7, after "NewSfi" insert --[SEQ ID No.7]--

IN THE CLAIMS:

Please cancel claims 1-49 and add claims 50-95.

--50. A signal amplification system comprising a bacterial multi-hybrid system of at least two chimeric polypeptides containing :

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(a) a first chimeric polypeptide corresponding to a first fragment of an enzyme;

(b) a second chimeric polypeptide corresponding to a second fragment of an enzyme or a modulating substance capable of activating said enzyme;

wherein the first fragment is fused to a molecule of interest and the second fragment or the modulating substance is fused to a target ligand and wherein the activity of the enzyme is restored by the *in vivo* interaction between the said molecule of interest and the said target ligand and wherein a signal amplification is generated; and

wherein signal amplification is performed in an *E. coli* strain selected from the group consisting of strain **BTH101** having C.N.C.M. Deposit Accession No. I-2309 and strain **DHM1** having C.N.C.M. Deposit Accession No. I-2310.

51. The signal amplification system according to claim 50, wherein the enzyme is an enzyme selected from the group consisting of adenylate cyclase and guanylate cyclase from any origin; or

the enzyme is the catalytic domain of *Bordetella* adenylate cyclase (CyaA), located within the first 400 amino acid residues of the adenylate cyclase enzyme.

52. The signal amplification system according to claim 51, wherein the first and the second fragments are any combination of fragments from the same enzyme, which *in vitro* functionally interact with the natural activator of said enzyme by restoring its activity.

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53. The signal amplification system according to claim 52, wherein the first and the second fragments are selected from the group consisting of :

(a) a fragment T25 corresponding to amino acids 1 to 224 of CyaA and a fragment T18 corresponding to amino acids 225 to 399 of CyaA;

(b) a fragment corresponding to amino acids 1 to 224 of CyaA and a fragment corresponding to amino acids 224 to 384 of CyaA;

(c) a fragment corresponding to amino acids 1 to 137 of CyaA and a fragment corresponding to amino acids 138 to 400 of CyaA;

(d) a fragment corresponding to amino acids 1 to 317 of CyaA and a fragment corresponding to amino acids 318 to 400 of CyaA ;

(e) two fragments from eukaryotic adenylate cyclase in association with molecules such as G protein, forskolin; or

(f) fragment T25 corresponding to amino acids 1 to 224 of *Bordetella pertussis* CyaA and a fragment T18 corresponding to amino acids 225 to 399 of *Bordetella pertussis* CyaA.

54. The signal amplification system according to claim 50, wherein the modulating substance is a natural activator, or a fragment thereof, of the enzyme.

55. The signal amplification system according to claim 54, wherein the natural activator is the calmodulin (CaM), or a fragment thereof, and said first fragment is mutated compared to the wild type enzyme.

56. The signal amplification system according to claim 55, wherein the first fragment is a mutated fragment of the catalytic domain of *Bordetella* adenylate cyclase (CyaA).

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57. A DNA library containing a collection of vectors transformed in a bacterial multi-hybrid system, wherein each vector contains a polynucleotide coding for the molecule of interest fused to a polynucleotide encoding for a first or second fragment of an enzyme.

58. DNA library according to claim 57, wherein the polynucleotide is selected from the group consisting of cDNA, RNA, genomic DNA, mitochondrial DNA.

59. DNA library according to claims 57, which is a H. *pylori* DNA library having C.N.C.M. Deposit Accession No. I-2367.

60. A method of selecting a molecule of interest, which is capable of binding to target ligand, wherein the interaction between the said molecule of interest and the said target ligand is detected with a signal amplification system according to claim 50, by means of generating a signal amplification and triggering transcriptional activation or repression, wherein the method of selecting the molecule of interest is performed in an *E. coli* strain selected from the group consisting of strain **BTH101** having C.N.C.M. Deposit Accession No. I-2309 and strain **DHM1** having C.N.C.M. Deposit Accession No. I-2310.

61. The method of selecting a molecule of interest according to claim 60, wherein the signal amplification corresponds to the production of a signaling molecule.

62. The method of selecting a molecule of interest according to claim 60, wherein the transcriptional activation leads to a reporter gene expression.

63. The method of selecting a molecule of interest according to claim 60, wherein the signal amplification system comprises a bacterial multi-hybrid system of at

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least two distinct fragments of an enzyme, whose enzymatic activity is restored by the interaction between the said molecule of interest and the said target ligand.

64. The method of selecting a molecule of interest according to claim 60, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least a first fragment of an enzyme and a modulating substance, whose activity is restored by the interaction between the said molecule of interest and the said target ligand.

65. The method of selecting a molecule of interest according to claim 60, wherein the target ligand is selected from the group consisting of protein, peptide, polypeptide, receptor, ligand, antigen, antibody, DNA binding protein, glycoprotein, lipoprotein, and recombinant protein.

66. The method of selecting a molecule of interest according to claim 60, wherein the molecule of interest is capable of interacting with the target ligand and possibly of binding to said target ligand.

67. The method of selecting a molecule of interest according to claim 60, wherein the interaction between the molecule of interest and the target ligand is detected by means of signal amplification which triggers transcriptional activation, and is quantified by measuring the synthesis of the signaling molecule or the expression of the reporter gene.

68. The method of selecting a molecule of interest according to claim 60, wherein the signaling molecule corresponds to the synthesis of cAMP.

69. The method of selecting a molecule of interest according to claim 63, wherein the signaling molecule corresponds to the synthesis of cGMP.

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70. The method of selecting a molecule of interest according to claim 62, wherein the reporter gene expression is selected from the group consisting of gene coding for nutritional marker such as lactose, maltose; gene conferring resistance to antibiotics such as ampicillin, kanamycin or tetracyclin; gene encoding for toxin; color marker such as fluorescent marker of the type of the Green Fluorescent Protein (GFP); gene encoding for phage receptor proteins or fragment thereof such as phage λ receptor, *lamB* and any other gene giving a selectable phenotype.

71. The method of selecting a molecule of interest according to claim 60, wherein the molecule of interest is a mutant molecule compared to a known wild type molecule and said molecule of interest is tested for its capacity of interacting with the target ligand.

72. The method of selecting a molecule of interest according to claim 60, wherein the selection is performed in strain **DHM1**.

73. The method of selecting a molecule of interest according to claim 60, wherein the selection is performed in strain **BTH101**.

74. A kit for selecting molecule of interest, wherein said kit comprises :

(a) a signal amplification system according to claim 60;

(b) a bacterial strain deficient in endogenous adenylate cyclase selected from the group consisting of strain **BTH101** having C.N.C.M. Deposit Accession No. I-2309 and strain **DHM1** having C.N.C.M. Deposit Accession No. I-2310;

(c) a medium allowing the detection of the complementation selected from the group consisting of indicator or selective medium as minimal medium supplemented with lactose or maltose as unique carbon source, medium with

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antibiotics, medium to visualize fluorescence, luminescense, conventional medium, such as xGAL medium, and medium which allows the sorting by the presence of the phage receptor.

75. A kit for selecting molecule of interest, wherein said kit comprises:

(a) a signal amplification system according to claim 60, wherein the molecule of interest is a mutant molecule compared to the known wild type molecule;

(b) a signal amplification system according to claim 60, wherein the molecule of interest is the known wild type molecule as the control;

(c) a bacterial strain deficient in endogenous adenylate cyclase selected from the group consisting of strain **BTH101** having C.N.C.M. Deposit Accession No. 1-2309 and strain **DHM1** having C.N.C.M. Deposit Accession No. I-2310;

(d) a medium allowing the detection of the complementation selected from the group consisting of indicator or selective medium as minimal medium supplemented with lactose or maltose as unique carbon source, medium with antibiotics, medium to visualize fluorescence, luminescence, conventional medium, such as xGAL medium, and medium which allows the sorting by the presence of the phage receptor for each signal amplification system;

(e) means for detecting whether the signal amplification system with the mutant molecule is enhanced or inhibited with respect to the signal amplification system with wild type.

76. A method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest wherein respectively the stimulating or the inhibiting activity is detected with a signal amplification system

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according to claim 60, by means of generating an amplification and respectively of triggering or of abolishing transcriptional activation;

wherein said signal amplification and said triggered or abolished transcriptional activation are compared with those obtained from an identical signal amplification system without any substance; and

wherein the method of screening for the substance is performed in an *E. coli* strain selected from the group consisting of strain **BTH101** having C.N.C.M. Deposit Accession No. I-2309 and strain **DHM1** having C.N.C.M. Deposit Accession No. I-2310.

77. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 75, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least two distinct fragments of an enzyme, whose enzymatic activity is restored by the interaction between the said molecule of interest and the said target ligand.

78. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 76, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least a first fragment of an enzyme and a modulating substance, whose activity is restored by the interaction between the said molecule of interest and the said target ligand.

79. The method of screening for substance capable of stimulating the interaction between a target ligand and a molecule of interest according to claim 76, wherein the signal amplification corresponds to the production of a signaling molecule.

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80. The method of screening for substance capable of inhibiting the interaction between a target ligand and a molecule of interest according to claim 76, wherein the signal amplification corresponding to the production of a signaling molecule is blocked or partially abolished.

81. The method of screening for substance capable of stimulating the interaction between a target ligand and a molecule of interest according to claim 76, wherein the transcriptional activation leads to a reporter gene expression.

82. The method of screening for substance capable of inhibiting the interaction between a target ligand and a molecule of interest according to claim 76, wherein the transcriptional activation leading to a reporter gene expression is blocked or partially abolished.

83. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 76, wherein the target ligand is selected from the group consisting of receptor, ligand, antigen, antibody, DNA binding protein, glycoprotein and lipoprotein.

84. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 76, wherein the substance is selected from the group consisting of protein, glycoprotein, lipoprotein, ligand and any other drug having stimulating or inhibitory affinity.

85. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 79, wherein the signaling molecule corresponds to the synthesis of cAMP.

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86. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 79, wherein the signaling molecule corresponds to the synthesis of cGMP.

87. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 81, wherein the reporter gene expression is selected from the group consisting of gene coding for nutritional marker such as lactose, maltose; gene conferring resistance to antibiotics such as ampicillin, kanamycin or tetracyclin; gene encoding for toxin; color marker such as fluorescent marker of the type of the Green Fluorescent Protein (GFP); gene encoding for phage receptor proteins or fragment thereof such as phage λ receptor, *lamB* and any other gene giving a selectable phenotype.

88. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 76, wherein the molecule of interest is a mutant molecule compared to the known wild type molecule and said molecule of interest is tested for its capacity of interacting with the target ligand.

89. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 76, wherein the screening is performed in strain **DHM1**.

90. The method of screening for a substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 76, wherein the screening is performed in strain **BTH101**.

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91. A kit for screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest, wherein said kit comprises:

(a) a signal amplification system according to claim 50 with the substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest;

(b) a signal amplification system according to claim 50 without any substance as the control;

(c) a bacterial strain deficient in endogenous adenylate cyclase selected from the group consisting of strain **BTH101** having C.N.C.M. Deposit Accession No. I-2309 and strain **DHM1** having C.N.C.M. Deposit Accession No. I-2310;

(d) a medium allowing the detection of the complementation selected from the group consisting of indicator plate or selective medium as minimal medium supplemented with lactose or maltose as unique carbon source, medium with antibiotics, medium to visualize fluorescence, conventional medium and medium which allows the sorting by the presence of the phage receptor and;

(e) means for detecting whether the signal amplification system with the substance is enhanced or inhibited with respect to the signal amplification system without any substance.

92. A molecule of interest identified by the method of claim 60.

93. A substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest identified by the method of claim 76.

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94. The signal amplification system according to claim 50, wherein the bacterial multi-hybrid system contains:

(a) a first chimeric polypeptide corresponding to a first fragment a of an enzyme;

(b) a second chimeric polypeptide corresponding to a second fragment of an enzyme or a modulating substance capable of activating said enzyme;

(c) a substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest, wherein the first fragment is fused to a molecule of interest and the second fragment or the modulating substance is fused to a target ligand and wherein the activity of the enzyme is restored by the interaction between the said molecule of interest and the said target ligand and wherein a signal amplification is generated; and

a bacterial strain deficient in endogenous adenylate cyclase selected from the group consisting of strain **BTH101** having C.N.C.M. Deposit Accession No. I-2309 and strain **DHM1** having C.N.C.M. Deposit Accession No. I-2310.

95. Strain **BTH101** having C.N.C.M. Deposit Accession No. I-2309, or Strain **DHM1** having C.N.C.M. Deposit Accession No. I-2310.--

REMARKS

Entry and consideration of this amendment is respectfully requested.

Claims 1-49 have been canceled. New claims 50-95 are derived from the original claims and find support throughout the specification. Accordingly, no new matter is entered by amendment.

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If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: August 23, 2001

By: _____

Kenneth J. Meyers
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